pieces on human primary OA osteoblasts was investigated using ALP assay.

Results: Subchondral BMD distribution was heterogeneous and displaying focal areas of relatively high mineralization density that spatially overlapped with areas of severe cartilage degeneration Contralateral tibial plateaus instead showed a homogeneous subchondral BMD of relatively low density. Histomorphometric analysis revealed an increase ($p < 0.001$) in subchondral bone area fraction (B.Ar/T.Ar.) in sclerotic (0.662 ± 0.120) compared with nonsclerotic tissue sections (0.335 ± 0.062). Corresponding with the observations at the macroscopic level, Mankin score and B.Ar/T.Ar were strongly positively correlated at a histological level ($r = 0.61$, $p < 0.001$). Immunohistological and flow cytometry analyses of subchondral bone marrow tissue showed a highly specific ($p < 0.001$) increase in CD68+ mononuclear and multinucleated cells and CD20+ B-lymphocytes in sclerotic compared with nonsclerotic subchondral bone tissue. Corresponding with an increase in multinucleated CD68+ cells, TRAP staining revealed a strong increase in functional osteoclasts that associated with CD34+ vascular structures. An increased number of TRAP+ macrophage-like mononuclear cells was observed in outgrowth cultures from sclerotic bone pieces. Overall, immune and TRAP+ cells were strongly correlated with B.Ar/T.Ar ($r = 0.804$ and $r = 0.670$, $p < 0.001$). Sclerotic OA osteoblasts showed poor in vitro mineralization and increased basal alkaline (ALP) phosphate activity. Lack of osteoblastic ALP induction by conditioned medium from sclerotic subchondral bone pieces suggested a proresorptive milieu in immune cell infiltrated marrow tissue.

Conclusion: We have shown for the first time that osteoimmunological mechanisms, including enhanced immune cell infiltration and osteoclast activity, along phenotypic alterations in osteoblasts are involved in uncoupled and aberrant bone remodeling underlying osteosclerosis in human OA.

200
PAPSS2 PROMOTES ALKALINE PHOSPHATES ACTIVITY AND MINERALIZATION OF OSTEOBLASTIC MC3T3-E1 CELLS BY CROSSTALK AND SMADS SIGNAL PATHWAYS

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Several studies have indicated that PAPSS2 (39-phosphoadenosine-59-phosphosulfate synthetase 2) activity is important to normal skeletal development. Mouse PAPSS2 is predominantly expressed during the formation of the skeleton and cartilaginous elements of the mouse embryo and in newborn mice. However, the role and mechanism of PAPSS2 in bone formation remains largely unidentified. By analyzing the expression pattern of the PAPSS2 gene, we have found that PAPSS2 is expressed in bone tissue and bone formation. PAPSS2 transcripts increase during osteoblast differentiation and are in less level in RANKL-induced osteoclast like cells. By using lentivirus-mediated RNA interference (RNAi) technology, we knocked down PAPSS2 expression in MC3T3-E1 osteoblast. Silencing of PAPSS2 expression significantly decreases ALP activity and cell mineralization, inhibits expression of osteoblast marker osteopontin (OPN) and collagen I. Conversely, overexpression of PAPSS2 promotes the MC3T3-E1 to differentiate into osteoblast and mineralization. Moreover, compared to that in the control cells, the mRNA level and protein expression of phosphorylated Smad 2/3, which is a key transcriptional factor in the Smad osteoblast differentiation pathway, showed significant decreases in PAPSS2-silenced cells and increases in PAPSS2- overexpression cells. These results suggest that PAPSS2 might regulate osteoblast ALP activity and cell mineralization, probably through Smads signal pathways.

201
VASCULAR PERFUSION OF THE PALMAR CONDYLES OF THE EQUINE THIRD METACARPAL BONE: A POSSIBLE CONTRIBUTING FACTOR TO PALMAR OSTEOCHONDRAL DISEASE?

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Purpose: Palmar osteochondral disease (POD) is an overload arthrosis that commonly affects fetlock joints of racing Thoroughbreds (TB), but the etiopathogenesis of the disease has not been well defined. The aim of this study was to compare India ink perfusion, as a measure of vascular perfusion, in the dorsal and palmar condyles of the third metacarpal bone (MC3) in both passively flexed (control group) and maximally extended (intervention group) fetlock joints from paired normal equine cadaver limbs. We hypothesized that the palmar condyle of MC3 normally has less perfusion than the dorsal condyle, and that loading the fetlock joint in maximal extension would result in further reduction of perfusion to the palmar condyles.

Methods: Pairs of forelimbs were acquired from 5 TB horses euthanized for reasons unrelated to lameness. Limb pairs were perfused intra-arterially with India ink (total of approximately 100 mL) within 1 hour post mortem. One forelimb from each horse was randomly assigned to either the passive flexion ($n = 5$) or maximal extension ($n = 5$) group. For the passive flexion group, limbs were allowed to assume a position of flexion without any external force applied. Limbs in the maximal extension group were placed in a materials testing system and the fetlocks extended to 110°, which has been reported to be maximal extension. Limbs were sectioned sagittally in 3 mm sections through the fetlock, and 12 sections per limb were processed using a modified tissue-clearing technique. Sections were subsequently digitally imaged, and bone perfusion evaluated with image analysis software. Elliptical regions of interest (ROIs) of standard size (3.47 mm x 8.34 mm) were digitally placed on the images in 2 locations: 1) on the subchondral bone just dorsal to the transverse ridge of MC3, and 2) on the subchondral bone just palmar to the transverse ridge (Fig 1). The palmar ROI was in a similar location to what has been reported for POD lesions (5–8 mm palmar to the transverse ridge).

Results: Greater perfusion of the dorsal condyle compared to the palmar condyle was observed in 78% of sections from limbs in passive flexion, and 92% of maximally extended sections (Fig 2). Perfusion to the palmar aspect of the condyle was significantly decreased ($P < 0.0001$) when the limbs were placed in maximal extension compared to passive flexion. The abaxial and mid sagittal ridge regions of MC3 appeared to have dorsal to palmar (D: P) perfusion ratios > 1 in the majority of samples, but did not appear to change based on whether the limb was in flexion or extension. Conversely, the medial axial to medial middle region of the condyle appeared to be the most vulnerable regions to maximal extension and actually had D:P ratios >1.5, thus illustrating the profound effect of maximal extension.

Conclusions: Our indirect evidence of relatively poor perfusion of the palmar condyles compared to the dorsal condyles gives some basis for considering these inherent differences in blood supply as a contributing factor for the pathogenesis of POD. During race training there is repetitive hyperextension of the fetlock joint, which likely further exacerbates this perfusion discrepancy. The locations on the palmar condyle that were most greatly affected by decreased India ink perfusion in our study were those locations where POD has been reported to occur most frequently. Although it was beyond the limits of this study to determine a cause and effect relationship between these observations, it does raise the possibility.

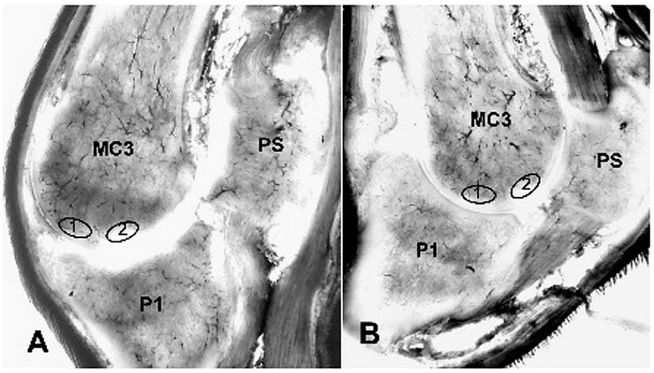

Fig 1. A: Representative sagittal section of the fetlock joint fixed in passive flexion. B: A representative sagittal section of the fetlock fixed in maximal extension. Ellipses illustrate region of interest (1-dorsal condyle; 2-palmar condyle) for calculation of mean pixel density of India ink perfusion. MC3 - third metacarpal bone; P1 - proximal phalanx; PS - proximal sesamoid bone.